



Effective Determination of Glutamic Acid by HPLC with ELSD

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Introduction

Glutamic acid or glutamate is one of the 20 most common natural amino acids. It possesses a carboxylic acid component to its side chain. Glutamic acid is critical for proper cell function, but is not considered an essential nutrient in humans because the body can manufacture it from simpler compounds. In addition to being one of the building blocks in protein synthesis, it is also important to brain function as an excitatory neurotransmitter. Free glutamic acid cannot cross the blood-brain barrier in appreciable quantities. Instead, it is converted into L-glutamine, which the brain uses for fuel and protein synthesis. The sodium salt of glutamic acid, monosodium glutamate (MSG) is responsible for one of the five basic tastes of the human sense of taste, and MSG is extensively used as a food additive. HPLC is often used in assays for the determination of glutamic acid. In this example, an isocratic HPLC method was employed to analyze DL-glutamic acid at low concentrations to determine the on-column limit of detection (LOD). A Varian evaporative light scattering (ELS) detector was employed because of its superior performance, as the detection method is independent of the optical properties of the compound under consideration.

Instrumentation

Column: C18 3 μ m, 150 x 4.6 mm

Detector: Varian ELSD (neb=50 °C, evap=70 °C, gas=1.2 SLM)

Materials and Reagents

Eluent: 95 % Water, 5 % Acetonitrile

Conditions

Flow Rate: 0.5 mL/min

Injection Volume: 10 μ L

Results and Discussion

In this case, the LOD is defined as a peak height of greater than three times the baseline noise. The chromatograms in Figure 1 illustrate the signal to noise ratio (S/N) obtained for injections of 200 ng and 100 ng on-column. The 100 ng loading gave a S/N=5, so for this application, the LOD is less than 100 ng on-column.

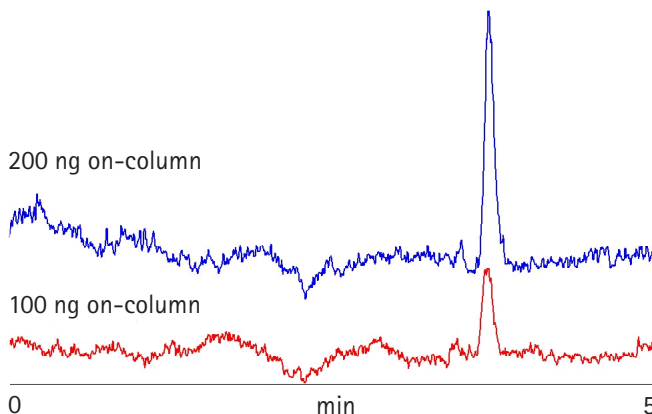


Figure 1. Very low signal to noise ratios achieved by Varian ELSD.

Conclusion

The Varian ELS detector successfully revealed an LOD for glutamic acid below 100 ng on-column. The Varian ELS detector surpasses other ELSDs for low temperature HPLC applications with semi-volatile compounds. Its innovative design represents the next generation of ELSD technology, providing optimum performance across a diverse range of HPLC applications. The Varian ELS detector's unique gas control permits evaporation of high boiling solvents at very low temperatures. For example, 100 % water at a flow rate of 5 mL/min can be removed at 30 °C. The novel design of the Varian ELS detector provides superior performance compared to detectors from other vendors for the analysis of semi-volatile compounds.

These data represent typical results. For further information, contact your local Varian Sales Office.

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